

Claims

1. An isolated and purified microbial organism, wherein said microbial organism is capable of fermenting malic acid to lactic acid,
- 5 and wherein said microbial organism when placed in a medium containing a predetermined amount of citric acid is only capable of degrading at the most 80% of said citric acid,
- 10 and wherein the microbial organism has at least one of the following characteristics, when said microbial organism in a frozen or freeze dried state is added directly into a fermented fruit juice:
- 15 i) a survival rate which is at least 1% after two days at 23°C in a fermented sterile fruit juice with a pH of less than 4 and comprising at least 12 vol% ethanol
- ii) a survival rate which is at least 70% after two days at 17°C in a fermented sterile fruit juice with a pH of less than 4 comprising at least 13.9 vol% ethanol
- 20 2. The microbial organism according to claim 1, wherein said microbial organism is an *Oenococcus oeni* strain.
- 25 3. The microbial organism according to claim 1, wherein the characteristic is a survival rate which is at least 1% after two days at 23°C in a wine prepared by yeasting a sterile grape fruit juice without added sulphite, said wine having an ethanol content of 12.0 vol%, pH 3.4, below 5 g/L residual sugar, 3.3 g/L of malic acid, and 450 mg/L of citric acid.
- 30 4. The microbial organism according to claim 1, wherein the characteristic is a survival rate which is at least 10% after two days at 23°C in a wine prepared by yeasting a sterile grape fruit juice without added sulphite, said wine having an ethanol content of 12.0 vol%, pH 3.4, below 5 g/L residual sugar, 3.3 g/L of malic acid, and 450 mg/L of citric acid.

- 5 5. The microbial organism according to claim 1, wherein the characteristic is a survival rate which is at least 45% after two days at 23°C in a wine prepared by
yeasting a sterile grape fruit juice without added sulphite, said wine having an ethanol content of 12.0 vol%, pH 3.4, below 5 g/L residual sugar, 3.3 g/L of malic acid, and 450 mg/L of citric acid.
- 10 6. The microbial organism according to claim 1, wherein the characteristic is a survival rate which is in the range of 70% to 100% after two days at 18°C in a wine prepared with 30 ppm SO₂ added before the alcoholic fermentation, said wine having an ethanol content of 13.8 vol%, pH 3.5, 1.3 g/L malic acid, and 340 mg/L of citric acid.
- 15 7. The microbial organism according to claim 1, wherein the characteristic is a survival rate which is at least 80% after two days at 17°C in a wine prepared without SO₂ added, said wine having an ethanol content of 13.9 vol%, pH 3.6, 1.7 g/L malic acid, and 320 mg/L of citric acid.
- 20 8. The microbial organism according to claim 1, wherein said microbial organism when placed in a liquid composition comprising a predetermined amount of malic acid is capable of degrading at least 90% of said malic acid.
- 25 9. The microbial organism according to claim 1, wherein said microbial organism is only capable of degrading at the most 50% of said citric acid.
- 30 10. The microbial organism according to claim 1, wherein said microbial organism is capable of reducing malic acid within 9 days to less than 1 g/L, such as 0,5 g/L, for example 0,1 g/L when added directly in a frozen or freeze dried state to a fermented fruit juice at a concentration of CFUs in the range of 1×10^6 to 5×10^7 per ml, wherein said fermented fruit juice is prepared by yeasting a sterile fruit juice without added sulphite resulting in a fermented fruit juice having an ethanol

content of 12.0 vol%, pH 3.4, below 5 g/L residual sugar, 3.3 g/L of malic acid, and 450 mg/L of citric acid.

- 5 11. The microbial organism according to claim 1, wherein said microbial organism reduces the citric acid content by less than 50% within 50 days, when added directly in a frozen or freeze dried state to a fermented fruit juice at a concentration of CFUs in the range of 1×10^6 to 5×10^7 per ml, wherein said fermented fruit juice is prepared by yeasting a sterile fruit juice without added sulphite resulting in a fermented fruit juice having an ethanol content of 12.0 vol%, pH 3.4, below 5 g/L residual sugar, 3.3 g/L of malic acid, and 450 mg/L of citric acid.
- 10 12. The microbial organism according to any of claims 10 and 11, wherein said microorganism is incubated with said fermented fruit juice at a temperature of around 23°C.
- 15 13. The microbial organism according to claim 1, wherein said organism is resistant to bacteriophages.
- 20 14. The microbial organism according to any of the preceding claims, wherein said organism retains its characteristics during propagation and concentration.
- 25 15. The microbial organism according to claim 1, wherein said organism is selected from the group consisting of strains deposited under the accession numbers DMS 15569, DMS 15570, and DSM 15571.
- 30 16. A method of preferentially degrading malic acid over citric acid in a liquid composition comprising malic acid and citric acid, said method comprising the steps of
- i) Providing a liquid composition comprising malic acid and citric acid;
 - ii) Providing a microbial organism according to any of claims 1 to 15, wherein said microbial organism has been frozen or freeze dried,
 - iii) Adding said freeze dried or frozen microbial organism directly to said liquid composition

54

- iv) incubating said liquid composition and said microbial organism under conditions which allow degradation of at least 70% of the malic acid,
- v) thereby obtaining a final liquid composition comprising less than 30% of the initial malic acid and at least 20% of the initial citric acid.

5

17. The method according to claim 16, wherein the liquid composition is fruit juice or fermented fruit juice.

10

18. The method according to claim 16, wherein the liquid composition is grape juice or fermented grape juice.

19. The method according to claim 16, wherein the liquid composition has a pH in the range of 2 to 5.

15

20. The method according to claim 16, wherein the liquid composition comprises in the range of 5 to 15 vol% ethanol.

21. The method according to claim 16, wherein the liquid composition comprises less than 10 g/l.

20

22. The method according to claim 16, wherein the liquid composition comprises in the range of 1 to 10 g/L malic acid.

23. The method according to claim 16, wherein the liquid composition comprises in the range of 50 to 2000 mg/L citric acid.

25

24. The method according to claim 16, wherein the final liquid composition comprises at least 50% of the initial citric acid.

30

25. The method according to claim 16, wherein the final liquid composition comprises less than 20% of the initial malic acid.

26. The method according to claim 16, wherein the microbial organism is added at a concentration of less than 5×10^7 CFU per ml of the liquid composition.

35

27. The method according to claim 16, wherein the microbial organism is added at a concentration of in the range of 1×10^6 to 5×10^7 CFUs per ml of the liquid composition.
- 5 28. The method according to claim 16, wherein the incubation conditions comprises incubation at a temperature in the range of 5 to 40°C.
29. The method according to claim 18, wherein the fermented grape juice is selected from the group consisting of red wines, white wines and sparkling wines.
- 10 30. The method according to any of claims 16 to 29, wherein step iv) comprises incubation for a longer period of time than required for obtaining the desired fermentation of malic acid.
- 15 31. The method according to claim 24, wherein step iv) comprises incubation for a longer period of time than required for completion of malolactic fermentation.
32. A method of inducing malolactic fermentation during wine production, comprising the steps of
- 20
- i) Providing a grape juice or a fermented grape juice
 - ii) Providing a microbial organism according to any of claims 1 to 15,
 - iii) Incubating said grape juice or fermented grape juice with said microbial organism under conditions which allow degradation of malic acid,
 - 25 iv) thereby inducing malolactic fermentation
33. The method according to claim 32, wherein said microbial organism is in a frozen or freeze-dried state.
- 30 34. The method according to claim 33, wherein said microbial organism in a frozen or freeze-dried state is added directly to said grape juice or a fermented grape juice.

35. The method according to claim 32, wherein the microbial organism is added at a concentration of less than 5×10^7 CFU per ml of the grape juice or a fermented grape juice.
- 5 36. The method according to claim 32, wherein the microbial organism is added at a concentration of in the range of 1×10^6 to 5×10^7 CFUs per ml of the grape juice or a fermented grape juice.
- 10 37. The method according to claim 32, wherein the grape juice or a fermented grape juice has a pH in the range of 2 to 5, such as 3 to 4.
- 15 38. The method according to claim 32, wherein the grape juice or a fermented grape juice comprises in the range of 5 to 15 vol% ethanol, such as 10 to 14 vol% ethanol, for example at least 12 vol% ethanol.
- 20 39. The method according to claim 32, wherein the grape juice or a fermented grape juice comprises less than 10 g/L, such as less than 7 g/L, for example less than 5 g/L sugar.
- 25 40. The method according to claim 32, wherein the grape juice or a fermented grape juice comprises in the range of 1 to 10 g/L, such as 2 to 5 g/L, for example 3 to 4 g/L malic acid.
- 30 41. The method according to claim 32, wherein the grape juice or a fermented grape juice comprises in the range of 50 to 2000 mg/L, such as 100 to 1000 mg/L, for example 200 to 800 mg/L, such as 400 to 500 mg/L citric acid.
42. The method according to claim 32, wherein the wine is selected from the group consisting of red wines, white wines and sparkling wines.
43. A concentrate of microbial organisms comprising or consisting of the microbial organism according to any of the claims 1 to 15, wherein said concentrate has a content of colony forming units being in the range of 10^9 to 10^{12} per g.

44. The concentrate according to claim 43, wherein said concentrate has been prepared by centrifugation.
- 5 45. The concentrate according to claim 43, wherein said microbial organism has been propagated in an adaptation medium comprising at least 6% sugar
46. The concentrate according to claim 45, wherein said adaptation medium comprises at least 5% glucose
- 10 47. The concentrate according to claim 45, wherein said adaptation medium comprises at least 5% fructose
48. The concentrate according to claim 45, wherein said adaptation medium comprises at least 3% glucose and at least 3% fructose
- 15 49. The concentrate according to any of claims 45 to 48, wherein said microbial organism has been propagated in said adaptation medium for at least 12 hours, such as at least 24 hours, for example around 48 hours.
- 20 50. A method of producing a microbial organism according to any of claims 1 to 15, wherein said method comprises the steps of
- i) Providing a microbial organism resistant to a pH below 5 and an ethanol concentration of at least 8%,
 - 25 ii) Subjecting said microbial organism to mutagenesis, thereby obtaining more than one different mutated microbial organism
 - iii) Selecting mutated microbial organisms capable of fermenting malic acid to lactic acid, wherein said microbial organism when placed in a medium containing a predetermined amount of citric acid is only capable of degrading at the most 80% of said citric acid, and wherein
 - 30 the microbial organism has at least one of the following characteristics, when said microbial organism in a frozen or freeze dried state is added directly into a fermented fruit juice:

58

- a) a survival rate which is at least 1% after two days at 23°C in a fermented sterile fruit juice comprising at least 12 vol% ethanol;
- b) a survival rate which is at least 70% after two days at 17°C in a fermented sterile fruit juice comprising at least 13.9 vol% ethanol

5

51. The method according to claim 50, wherein said microbial organism is resistant to pH 3.2.

10 52. The method according to claim 50, wherein said microbial organism is resistant to an ethanol concentration of 13 vol%.

53. The method according to claim 50, wherein mutagenising comprises incubation in the presence of a mutagenising agent.

15

54. The method according to claim 53, wherein said mutagenising agent is selected from the group consisting of ethylmethanesulfonate, N-ethyl-N'-nitro-N-nitrosoguanidine, and 1-(2-hydroxyethyl)-1-nitrosourea.

20 55. A method of preparing a dried microbial organism capable of fermenting malic acid to lactic acid, which has reduced citric acid degrading activity and which is capable of survival after direct inoculation into fermented fruit juice, said method comprising the steps of

- 25 i) Providing a microbial organism according to any of claims 1 to 15,
- ii) Providing an adaptation medium comprising at least 6% sugar
- iii) Propagating said microbial organism in said adaptation medium under conditions allowing growth of said microbial organism
- 30 iv) Harvesting said microbial organism
- v) drying said microbial organism

56. The method according to claim 55, wherein said adaptation medium comprises at least 5% glucose

35

59

57. The method according to claim 55, wherein said adaptation medium comprises at least 5% fructose
58. The method according to claim 55, wherein said adaptation medium comprises at least 3% glucose and at least 3% fructose
59. The method according to claim 55, wherein said microbial organism is propagated in said adaptation medium for at least 12 hours, such as at least 24 hours, for example around 48 hours.
60. The method according to claim 55, wherein said conditions comprises propagated at a temperature in the range of 15 to 40°C, preferably around 30°C.
61. The method according to claim 55, wherein harvesting said microbial organism comprises centrifugation.
62. The method according to claim 55, wherein drying is freeze-drying.
63. An activation solution comprising
- i) A nitrogen source
 - ii) In the range of 60 to 140 g sugar per L
 - iii) In the range of 5×10^8 and 5×10^{10} colony forming units per ml of a microbial organism capable of fermenting at least one fermentable compound
64. The activation solution according to claim 63, wherein said fermentable compound is malic acid.
65. The activation solution according to claim 63, wherein said microbial organism is capable of fermenting malic acid to lactic acid.
66. The activation solution according to claim 63, wherein said microbial organism is selected from the group consisting of bacteria belonging to the *Oenococcus* family and the *Lactobacillus* family.

67. The activation solution according to claim 63, wherein said microbial organism is selected from the group consisting *Oenococcus oeni* and *Lactobacillus plantarum*.
- 5 68. The activation solution according to claim 63, wherein the microbial organism is selected from the group consisting of DSM 15568, DSM 7008, DMS 15569, DMS 15570, and DSM 15571.
- 10 69. The activation solution according to claim 63, wherein said microbial organism is selected from the group consisting of MBR Alpha, MBR Beta, MBR 31, MBR 41, MBR OSU, Inobacter (IB), OSU, ProVino, MCW, 3X, MT01, Viniflora oenos and Viniflora CH35.
- 15 70. The activation solution according to claim 63, wherein the sugar is selected from the group consisting of fructose and glucose
71. The activation solution according to claim 63, wherein the solution comprises in the range of 30 g to 100 g glucose.
- 20 72. The activation solution according to claim 63, wherein the solution comprises in the range of 30 g to 100 g fructose.
73. The activation solution according to claim 63, wherein the solution comprises a chemical compound with buffering capacity.
- 25 74. The activation solution according to claim 73, wherein the solution has a pH in the range of 4.0 to 6.0.
- 30 75. The activation solution according to claim 73, wherein said chemical compound is selected from the group consisting of tartaric acid, malic acid, lactic acid, phosphate and citrate.
76. A dry activation composition, wherein in the range of 80 to 200 g of said dry activation composition comprises
- 35 i) a nitrogen source

61

- ii) In the range of 60 to 140 g sugar
- iii) In the range of 5×10^{11} and 5×10^{13} colony forming units of a microbial organism capable of fermenting a fermentable compound, wherein addition of 1 L water to said dry activation compositions results in an activation solution according to any of claims 63 to 75

5

77. The composition according to claim 76, wherein in the range of 110 to 150 g of said dry activation composition comprises in the range of 80 to 110 g, such as 90 to 110 g, for example 95 to 105 g sugar.

10

78. The composition according to any of claims 76 to 77, wherein the sugar is selected from the group consisting of fructose and glucose

79. The composition according to any of claims 76 to 77, wherein the composition comprises in the range of 30 g to 100 g, such as 40 to 80 g glucose.

15

80. The composition according to any of claims 76 to 77, wherein the composition comprises in the range of 30 g to 100 g, such as 40 to 80 g fructose.

81. The composition according to any of claims 76 to 77, wherein the composition comprises in the range 40 to 80 g glucose and in the range of 40 to 80 g fructose.

20

82. The composition according to claim 76, wherein the fermentable compound is malic acid.

25

83. The composition according to claim 76, wherein said microbial organism when incubated in a liquid composition comprising a predetermined amount of malic acid is capable of fermenting at least 50% of said malic acid.

30

84. The composition according to claim 76, wherein said microbial organism is selected from the group consisting of bacteria belonging to the *Oenococcus* family and the *Lactobacillus* family.

85. The composition according to claim 76, wherein said microbial organism is selected from the group consisting *Oenococcus oeni* and *Lactobacillus plantarum*.
86. The composition according to claim 76, wherein the microbial organism is selected from the group consisting of DSM 15568, DSM 7008, DMS 15569, DMS 15570, and DSM 15571.
87. The composition according to claim 76, wherein the microbial organism is selected from the group consisting of MBR Alpha, MBR Beta, MBR 31, MBR 41, MBR OSU, Inobacter (IB), OSU, ProVino, MCW, 3X, MT01, Viniflora oenos and Viniflora CH35.
88. The composition according to claim 76, wherein the microbial organism after activation for more than 5 hours in the activation solution has a survival rate which is at least 3% after two days at 23°C when inoculated into a fermented fruit juice having an ethanol content of at least 12.0 vol%.
89. The composition according to claim 88, wherein activation is in the range of 8 to 48 hours.
90. The composition according to claim 88, wherein activation is performed at a temperature in the range of 18°C to 25°C.
91. The composition according to claim 88, wherein the survival rate is at least 3% after two days at 23°C when inoculated into a fermented fruit juice prepared by yeasting a sterile grape fruit juice without added sulphite, said fermented fruit juice having an ethanol content of 12.0 vol%, pH 3.4, below 5 g/L residual sugar, 3.3 g/L of malic acid, and 450 mg/L of citric acid
92. The composition according to claim 88, wherein the survival rate is at least 33% after two days at 23°C when inoculated into a fermented fruit juice prepared by yeasting a sterile grape fruit juice without added sulphite, said fermented fruit juice having an ethanol content of 12.0 vol%, pH 3.4, below 5 g/L residual sugar, 3.3 g/L of malic acid, and 450 mg/L of citric acid

93. The composition according to claim 88, wherein the survival rate is at least 94% after two days at 23°C when inoculated into a fermented fruit juice prepared by yeasting a sterile grape fruit juice without added sulphite, said fermented fruit juice having an ethanol content of 12.0 vol%, pH 3.4, below 5 g/L residual sugar, 3.3 g/L of malic acid, and 450 mg/L of citric acid
94. The composition according to claim 76, wherein the composition comprises a chemical compound with buffering capacity.
95. The composition according to claim 94, wherein the chemical compound is capable of buffering a solution to a pH in the range of 4.0 to 6.0.
96. The composition according to claim 94, wherein said chemical compound is selected from the group consisting of tartaric acid, malic acid, lactic acid, phosphate and citrate.
97. The composition according to claim 76, wherein said composition furthermore comprises a salt.
98. A method of inducing fermentation in a liquid composition comprising a fermentable compound comprising the steps of
- i) Providing a dry composition according to any of claims 76 to 97, wherein said microbial organism is capable of fermenting said fermentable compound
 - ii) Adding water to said dry composition, thereby obtaining an activation solution
 - iii) Incubating said activation solution for an activation time under activation conditions
 - iv) Providing a liquid composition comprising said fermentable compound
 - v) Inoculating said liquid composition with said activation solution
 - vi) Thereby inducing fermentation in said liquid composition

99. The method according to claim 98, wherein the liquid composition is a fruit juice or a fermented fruit juice.

5 100. The method according to claim 98, wherein the liquid composition is grape fruit juice or fermented grape fruit juice.

101. The method according to claim 98, wherein activation time is at least 5 hours.

10 102. The method according to claim 98, wherein the activation time is in the range of 8 to 48 hours.

15 103. The method according to claim 98, wherein activation conditions comprises incubation at a temperature in the range of 10°C to 40°C, such as 18°C to 25°C.

104. The method according to claim 98, wherein the fermentable compound is malic acid.